Fish & Shellfish Immunology 26 (2009) 368–376

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Early treatment with *Lactobacillus delbrueckii* strain induces an increase in intestinal T-cells and granulocytes and modulates immune-related genes of larval *Dicentrarchus labrax* (L.)

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ARTICLE INFO

Article history:
Received 23 July 2008
Received in revised form
30 September 2008
Accepted 13 October 2008
Available online 1 November 2008

Keywords:
Lactobacillus delbrueckii delbrueckii
Probiotics
Dicentrarchus labrax
GALT
larval immunity
T-cells
Cytokines

ABSTRACT

Lactobacillus delbrueckii ssp. *delbrueckii* (AS13B), isolated from the gut of adult *Dicentrarchus labrax*, was administered live to developing sea bass using rotifers and *Artemia* as live carriers. Immune-related gene transcripts were quantified in post-larvae at day 70 post-hatch (ph) and histology, electron microscopy and immunocytochemistry of the intestinal tissue were performed at day 74 ph. Since the probiotic was orally administered the studies were focused on intestinal immunity.

In treated fish gut integrity was unaffected, while the density of T-cells and acidophilic granulocytes in the intestinal mucosa was significantly higher than in controls. Probiotic-induced increases in intestinal T-cells and total body *TcR-β* transcripts are first reported in fish. Significantly lower *IL-1β* transcripts and a trend towards lower *IL-10*, *Cox-2* and *TGF-β* transcription were found in the treated group.

Evidence is provided that early feeding with probiotic-supplemented diet stimulated the larval gut immune system and lowered transcription of key pro-inflammatory genes.

1. Introduction

The European sea bass, *Dicentrarchus labrax* (L.), is the most intensively farmed marine fish in south Europe. However, numerous viruses, bacteria, fungi and parasites affect the species, causing infectious diseases, and thereby leading to heavy losses in aquaculture production. These problems particularly arise during the larval stage, the most critical period of rearing. The use of antimicrobial agents causes environmental concerns, and their effectiveness in preventing or controlling fish diseases has been questioned, given extensive documentation on the evolution of drug resistance by pathogenic bacteria.

The use of probiotics or beneficial bacteria in animal nutrition is well documented and has recently begun to be applied in aquaculture [1,2] as an alternative to antibiotics. *Lactobacilli* and *Bifidobacteria* can be used as probiotics since, when exogenously administered, they can exert benefits on health other than nutritional support [3]. The mechanisms of action of probiotics are far from completely understood, although it is widely accepted that they can produce inhibitory compounds, compete against pathogenic bacteria for nutrients and adhesion sites, improve the microbial balance, and modulate the physiology of the immune system [4]. Numerous microorganisms have been used as probiotics to improve growth or survival of larval aquatic species [5–8]. It has been suggested that the efficacy of probiotics is highest in the host species from which they were isolated [9], therefore candidate aquatic probionts for larviculture were isolated from healthy adults [10,11] and larvae [6,12]. However, even the human probiotic *Lactobacillus rhamnosus* enhanced survival of rainbow trout challenged with a virulent strain of *Aeromonas salmonicida* [13], and some probiotics used for human beings and terrestrial animals have given promising results in aquaculture species [7,13–16].

Abbreviations: AGs, acidophilic granulocytes; GALT, gut-associated lymphoid tissue; IEL, intraepithelial lymphocytes; LAB, lactic acid bacteria; LP, lamina propria; mAb, monoclonal antibody; MGG, May-Grünwald/Giemsa; ph, post-hatch; TEM, transmission electron microscopy.

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doi:10.1016/j.fsi.2008.10.008